# NICOTINIC ACETYLCHOLINE RECEPTOR PEPTIDE ANTAGONIST CONOTOXIN COMPOSITIONS AND RELATED METHODS

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application Serial No. 62/746,398 filed October 16, 2018, and U.S. Provisional Application Serial No. 62/867,128 filed June 26, 2019, which are incorporated herein by reference in their entirety.

## SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 9, 2019, is named 54780-703_601_SL.txt and is 30,738 bytes in size.

## BACKGROUND OF THE INVENTION

**[0003]** Muscle-type nicotinic acetylcholine receptors in vertebrate skeletal muscles mediate neuromuscular transmission at the neuromuscular junction. They respond to the binding of acetylcholine to cause muscle contraction.

## SUMMARY

**[0004]** The present disclosure includes compositions and methods relating to novel peptide antagonists that inhibit binding of acetylcholine (ACh) to the active site of the muscle-type nicotinic acetylcholine receptor (muscle nAChR). In some examples, the disclosure includes cosmetic or pharmaceutical compositions comprising a peptide antagonist of the disclosure. In some examples, the peptide antagonists of the disclosure are useful in topical cosmetic or pharmaceutical compositions for preventing or improving of the appearance of skin wrinkles or related skin conditions. In some examples, the disclosure relates to pharmaceutical compositions for preventing or treating a disorder ameliorated by inhibition of muscle nAChR, e.g., migraine headache. In some examples, the disclosure relates to methods of using a cosmetic or pharmaceutical composition comprising a peptide antagonist of the disclosure. In some examples, the disclosure relates to methods for blocking or inhibiting neuromuscular transmission and/or ACh binding to muscle nAChR using a compositions or method of the disclosure.

**[0004a]** In one aspect, the present disclosure refers to a muscle-type nicotinic acetylcholine receptor peptide antagonist comprising an amino acid sequence:

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-Xaa12-Xaa13-Xaa14

wherein:

Xaa1 is absent;

Xaa2 is absent;

Xaa3 and Xaa8 form a linkage Xaa3-Xaa8;

Xaa4 and Xaa14 form a linkage Xaa4-Xaa14;

Xaa5 is selected from: Asp, Gln, Glu, Arg, His and Lys;

Xaa6 is selected from: Pro and hydroxyproline;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Asn, Asp, Gln, Glu, Arg, His and Lys;

Xaa12 is selected from: Trp and Tyr;

Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, Arg, His and Lys;

the N-terminus is optionally modified; and

the C-terminus is optionally modified.

**[0004b]** In another aspect, the present disclosure refers to a cosmetic composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist as described here, for prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, in a subject.

**[0004c]** In another aspect, the present disclosure refers to a pharmaceutical composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist as described herein, for: prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity; treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus, in a subject.

**[0004d]** In yet another aspect, the present disclosure refers to a method for preventing or temporarily improving the appearance in a subject for prevention or temporary improvement of

the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, comprising applying an effective amount of the cosmetic composition as described herein to the skin of the subject.

**[0004e]** In yet another aspect, the present disclosure refers to a use of the pharmaceutical composition as described herein in the manufacture of a medicament for preventing or temporarily improving one or more of the appearance of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity; treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus; in a subject, wherein the pharmaceutical composition as described herein is to be administered an effective amount to the subject.

**[0004f]** In yet another aspect, the present disclosure refers to the pharmaceutical composition as described herein for use as a therapy.

**[0005]** The present disclosure includes a muscle-type nicotinic acetylcholine receptor peptide antagonist comprising an amino acid sequence Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-Xaa12-Xaa13-Xaa14, wherein: Xaa1 is absent or selected from Ala,

Gly,Val, Leu, Ile and a derivative of Ala, Gly,Val, Leu, or Ile; Xaa2 is absent or selected from: Asn, Asp, Gln, Glu, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, Ile, and a derivative of Asn, Asp, Gln, Glu, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, or Ile; Xaa3 and Xaa8 form a linkage Xaa3-Xaa8; Xaa4 and Xaa14 form a linkage Xaa4-Xaa14; Xaa5 is selected from: Asn, Asp, Gln, Glu, Arg, His, Lys, and a derivative of Asn, Asp, Gln, Glu, Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Asn, Asp, Gln, Glu, Arg, His, Lys, and a derivative of Asn, Asp, Gln, Glu, Arg, His, or Lys; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, Arg, His, Lys, and a derivative of Cys, Met, Sec, Ser, Thr, Arg, His, or Lys; the N-terminus is optionally modified; and the C-terminus is optionally modified.

**[0006]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is absent; the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Asn, Asp, Gln, Glu, and a derivative of Asn, Asp, Gln, or Glu; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, and a derivative of Cys, Met, Sec, Ser, or Thr.

**[0007]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is absent; the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly,

Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Asn, Asp, Gln, Glu, and a derivative of Asn, Asp, Gln, or Glu; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, and a derivative of Cys, Met, Sec, Ser, or Thr.

**[0008]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Asn, Asp, Gln, Glu, and a derivative of Asn, Asp, Gln, or Glu; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, and a derivative of Cys, Met, Sec, Ser, or Thr.

**[0009]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is absent; the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, and a derivative of Cys, Met, Sec, Ser, or Thr.

**[0010]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth,

wherein: Xaa1 is absent or selected from Ala, Gly,Val, Leu, Ile and a derivative of Ala, Gly,Val, Leu, or Ile; Xaa2 is absent or selected from: Asn, Asp, Gln, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, Ile, and a derivative of Asn, Asp, Gln, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, or Ile; the Xaa3-Xaa8 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Asp, Gln, Glu, Arg, His, Lys, and a derivative of Asp, Gln, Glu, Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Gly, Val, Leu, Ile and a derivative of Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Val, Leu, Ile and a derivative of Ala, Val, Leu, or Ile; Xaa10 is selected from: His, Lys, and a derivative of His or Lys; Xaa11 is selected from: Asn, Asp, Gln, Glu, Arg, Lys, and a derivative of Asn, Asp, Gln, Glu, Arg, or Lys; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe or Trp; and Xaa13 is selected from: Cys, Met, Sec, Thr, Arg, His, Lys, and a derivative of Cys, Met, Sec, Thr, Arg, His, or Lys.

**[0011]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent or selected from Ala, Gly,Val, Leu, Ile and a derivative of Ala, Gly,Val, Leu, or Ile; Xaa2 is absent or selected from: Asn, Asp, Gln, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, Ile, and a derivative of Asn, Asp, Gln, Arg, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, or Ile; the Xaa3-Xaa8 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Asp, Gln, Glu, Arg, Lys, and a derivative of Asp, Gln, Glu, Arg, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Gly, Val, Leu, Ile and a derivative of Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Val, Leu, Ile and a derivative of Ala, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, and a derivative of Arg or Lys; Xaa11 is selected from: Asn, Asp, Gln, Glu, Arg, Lys, and a derivative of Asn, Asp, Gln, Glu, Arg, or Lys; Xaa12 is selected from: Trp, Tyr, and a derivative of Trp or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Thr, Arg, His, Lys, and a derivative of Cys, Met, Sec, Thr, Arg, His, or Lys.

**[0012]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent or selected from Ala, Val, Leu, Ile and a derivative of Ala, Val, Leu, or Ile; Xaa2 is absent or selected from: Asn, Asp, Gln, Glu, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val,

Leu, Ile, and a derivative of Asn, Asp, Gln, Glu, His, Lys, Phe, Trp, Tyr, Ala, Gly, Val, Leu, or Ile; the Xaa3-Xaa8 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Asn, Asp, Gln, Glu, Arg, Lys, and a derivative of Asn, Asp, Gln, Glu, Arg, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Gly, Val, Leu, Ile and a derivative of Gly,Val, Leu, or Ile; Xaa9 is selected from: Ala, Val, Leu, Ile and a derivative of Ala, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, and a derivative of Arg or His; Xaa11 is selected from: Asp, Gln, Glu, Arg, His, Lys, and a derivative of Asp, Gln, Glu, Arg, His, or Lys; Xaa12 is selected from: Phe, Trp, and a derivative of Phe or Trp; and Xaa13 is selected from: Cys, Met, Sec, Thr, Arg, His, Lys, and a derivative of Cys, Met, Sec, Thr, Arg, His, or Lys.

**[0013]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is absent or selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage; Xaa5 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa9 is selected from: Ala, Gly, Val, Leu, Ile and a derivative of Ala, Gly, Val, Leu, or Ile; Xaa10 is selected from: Arg, His, Lys, and a derivative of Arg, His, or Lys; Xaa11 is selected from: Arg, His, Lys, Asn, Asp, Gln, Glu, and a derivative of Arg, His, Lys, Asn, Asp, Gln, or Glu; Xaa12 is selected from: Phe, Trp, Tyr, and a derivative of Phe, Trp, or Tyr; and Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, and a derivative of Cys, Met, Sec, Ser, or Thr.

**[0014]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist as initially set forth, wherein: Xaa1 is absent; Xaa2 is absent or selected from: Arg and a derivative of Arg; the Xaa3-Xaa8 linkage is a Cys-Cys linkage; the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage and a cystathionine linkage; Xaa5 is selected from: His and a derivative of His; Xaa6 is selected from: Pro and a derivative thereof; Xaa7 is selected from: Ala and a derivative of Ala; Xaa9 is selected from: Gly and a derivative of Gly; Xaa10 is selected from: Arg, Lys, and a derivative of

Arg or Lys; Xaa11 is selected from: His, Asn, and a derivative of His or Asn; Xaa12 is selected from: Tyr and a derivative of Tyr; and Xaa13 is selected from: Ser and a derivative of Ser.

**[0015]** In some examples, the Xaa3-Xaa8 linkage and the Xaa4-Xaa14 linkage are independently selected from: a Cys-Cys linkage; a Sec-Sec linkage; a cystathionine linkage; a lactam bridge, a thioether linkage, and a dicarba linkage. In some examples, the thioether linkage is a lanthionine linkage. In some examples, the linkage between Xaa3 and Xaa8 has a spatial separation between the alpha carbons or the geometric centers of each of Xaa3 and Xaa8 of about 3.5 to about 10 angstroms, wherein the linkage between Xaa4 and Xaa14 has a spatial separation between the alpha carbons or the geometric centers of each of Xaa4 and Xaa14 of about 3.5 to about 10 angstroms, or both.

**[0016]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist of the present disclosure, wherein the N-terminus is modified to comprise $C_1$-$C_6$ acyl, $C_1$-$C_8$ alkyl, $C_6$-$C_{12}$ aralkyl, $C_5$-$C_{10}$ aryl, $C_4$-$C_8$ heteroaryl, formyl, or a lipid. In some examples, the N-terminus is not modified with an amino acid or a derivative of an amino acid.

**[0017]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist of the presentdisclosure, wherein the C-terminus is modified to comprise $NH_2$, amino-acyl, amino-$C_1$-$C_8$ alkyl, amino-$C_6$-$C_{12}$-aralkyl, amino-$C_5$-$C_{10}$ aryl, amino-$C_4$-$C_8$ heteroaryl, or O-($C_1$-$C_8$ alkyl). In some examples, the C-terminus is not modified with an amino acid or a derivative of an amino acid.

**[0018]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist of the present disclosure, wherein a lipid is covalently attached to a cysteine, serine, lysine, threonine or tyrosine. In some examples, the lipid is covalently attached to a cysteine or a lysine. In some examples, the lipid comprises a $C_6$-$C_{20}$ alkyl, $C_6$-$C_{20}$ alkenyl, $C_6$-$C_{20}$ alkynyl, or $C_6$-$C_{20}$ acyl group. In some examples, the lipid comprises a geranyl, farnesyl, or geranylgeranyl group. In some examples, the lipid comprises a undecyloyl, lauroyl, tridecyloyl, myristoyl, palmitoyl, or stearoyl group. In some examples, the lipid is a covalent modification of Cys added by palmitoylation or prenylation.

**[0019]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist of the present disclosure comprising at least one derivative that is a non-canonical amino acid selected from the group consisting of: an aromatic side chain amino acid; a non-aromatic side chain amino acid; an

aliphatic side chain amino acid; a side chain amide amino acid; a side chain ester amino acid; a heteroaromatic side chain amino acid; a side chain thiol amino acid; a beta amino acid; and a backbone-modified amino acid. In some examples, the aromatic side chain amino acid is a derivative of tyrosine, histidine, tryptophan, or phenylalanine. In some examples, the non-aromatic side chain amino acid is a derivative of serine, threonine, cysteine, methionine, arginine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, proline, glycine, alanine, valine, isoleucine, or leucine. In some examples, the at least one derivative is a non-canonical amino acid selected from the group consisting of: 2-aminoadipic acid; 3-aminoadipic acid; beta-alanine; beta-aminoproprionic acid; 2-aminobutyric acid; 4-aminobutyric acid; piperidinic acid; 6-aminocaproic acid; 2-aminoheptanoic acid; 2-aminoisobutyric acid; 3-aminoisobutyric acid; 2-aminopimelic acid; 2,4-diaminobutyric acid; desmosine; 2,2'-diaminopimelic acid; 2,3-diaminoproprionic acid; N-ethylglycine; N-ethylasparagine; hydroxylysine; allo-hydroxylysine; 3-hydroxyproline; 4-hydroxyproline; isodesmosine; allo-isoleucine; N-methylglycine; sarcosine; n-methylisoleucine; 6-N-methyllysine; N-methylvaline; norvaline; norleucine; and ornithine.

**[0020]** In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is the muscle-type nicotinic acetylcholine receptor peptide antagonist of the present disclosure, wherein one or more amino acids in the peptide have the D-amino acid configuration and the remaining amino acids in the peptide have the L-amino acid configuration.

**[0021]** In some examples, a muscle-type nicotinic acetylcholine receptor peptide antagonist of the disclosure selectively inhibits a muscle-type nicotinic acetylcholine receptor. In related examples, the $IC_{50}$ is about 1 millimolar to about 1 picomolar. In some examples, the $IC_{50}$ is determined by an activity assay. In some examples, the activity assay is a competitive binding assay or an assay of inhibition of the acetylcholine receptor ion channel conductance.

**[0022]** In some examples, the disclosure includes a cosmetic composition comprising a muscle-type nicotinic acetylcholine receptor peptide antagonist of the disclosure, for prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, in a subject. In some examples, the cosmetic composition is formulated for topical use. In some examples, the cosmetic composition comprises one or more excipient selected from the group consisting of: water, a buffer, an absorption enhancer, a stability enhancer, diaminobutyroyl benzylamide, diacetate, glycerin, a gum, a hydrophilic colloid or derivative, a cellulosic derivative, an emulsifier, a fatty alcohol, an acrylic derivative, a mineral, a surfactant, a fat, an oil, a

preservative, a monosaccharide, a disaccharide, a polysaccharide, a glycosaminoglycan, and a chelating agent. In some examples, the absorption enhancer is selected from the group consisting of: a liposome delivery system, a transfersome delivery system, an ethosome delivery system, a short chain alcohol, a long chain alcohol, a polyalcohol, urea, an amino acid, an amino acid ester, an amine, an amide, 1-dodecylazacycloheptan-2-one (AZONE®), a derivative of 1-dodecylazacycloheptan-2-one, a pyrrolidone, a pyrrolidone derivative, a terpene, a terpene derivative, a fatty acid, a fatty acid ester, a macrocyclic compound, a tenside, a sulfoxide, lecithin vesicles, water surfactants, a polyol, a small molecule tri, tetra, penta, hexa, septa or octa peptide, isoceteth-20, ethoxydiglycol, dimethyl sulfoxide, dimethyl isosorbide, and phloretin. In some examples, the stability enhancer is a small molecule peptide. In some examples, the small molecule peptide is selected from the group consisting of: a tripeptide, a tetrapeptide, a pentapeptide, a hexapeptide, a septapeptide, an octapeptide, Acetyl Hexapeptide-3 Cosmetic Topical Peptide, Melanotan II, ACVR2B (ACE-031), Argireline® Acetate, Argireline, Matrixyl Acetate (palmitoyl pentapeptide), peptide GHK spontaneously complexes with copper, Palmitoyl Tetrapeptide-3, Argireline, Acetyl Glutamyl Heptapeptide, Matrixyl™, Snap-8, Syn®-Tacks, Syn®-Coll, Syn®-Hycan, Leuphasyl®, Pepha®-Tight, Tego® Pep 4-17 and Trylagen®. In some examples, the cosmetic composition further comprises one or more other active ingredient. In some examples, the one or more other active ingredient is selected from the group consisting of: a second, different, muscle-type nicotinic acetylcholine receptor peptide antagonist, an antioxidant, a retinoid, a growth factor, a collagen stimulating peptide, a carrier peptide, a peptide that inhibits tTAT-superoxide dismutase, a peptide that inhibits a proteinase, a peptide that stimulates hyaluronan synthase 2, and a keratin-based peptide. In some examples, the cosmetic composition comprises a liposome delivery system. In some examples, the cosmetic composition is a cream, balm, gel, solution, serum, cosmetic, liquid, lotion, ointment, emulsion, milk, spray, mask, or the like. In some examples, the cosmetic composition comprises about 0.01% to about 5% w/w of the muscle-type nicotinic acetylcholine receptor peptide antagonist.

**[0023]** In some examples, the disclosure includes a cosmetic composition comprising a muscle-type nicotinic acetylcholine receptor peptide antagonist of the disclosure, for: prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity;

treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus, in a subject. In some examples, the pharmaceutical composition is formulated for intradermal, subcutaneous, intramuscular, or intradetrusor administration. In some examples, the pharmaceutical composition comprises one or more excipient.

**[0024]** In some examples, the disclosure includes a method for preventing or temporarily improving the appearance in a subject for prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, comprising applying an effective amount of the cosmetic composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist of the disclosure to the subject. In some examples of this method, the muscle-type nicotinic acetylcholine receptor peptide antagonist is applied in one or more doses of the cosmetic composition. In some examples, a single dose of the cosmetic composition is applied about once per hour to about once per 2 weeks. In some examples, a single dose of the cosmetic composition is applied about once per day. In some examples, the cosmetic composition can be applied indefinitely with no adverse effect. In some examples, the dose of the cosmetic composition is a subimmunological dose.

**[0025]** The disclosure further includes a method for: preventing or temporarily improving one or more of the appearance of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity; treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus; in a subject, comprising administering an effective amount of the pharmaceutical composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist of the disclosure to the subject. In some examples, the muscle-type nicotinic acetylcholine receptor peptide antagonist is administered in one or more treatments with the pharmaceutical composition. In some examples, administration of each treatment comprises injection of the pharmaceutical composition by injection into one or more treatment sites. In some examples, each treatment comprises a subimmunological dose of the pharmaceutical composition.

## CLAIMS

WHAT IS CLAIMED IS:

1. A muscle-type nicotinic acetylcholine receptor peptide antagonist comprising an amino acid sequence:

Xaa1-Xaa2-Xaa3-Xaa4-Xaa5-Xaa6-Xaa7-Xaa8-Xaa9-Xaa10-Xaa11-Xaa12-Xaa13-Xaa14

wherein:

Xaa1 is absent;

Xaa2 is absent;

Xaa3 and Xaa8 form a linkage Xaa3-Xaa8;

Xaa4 and Xaa14 form a linkage Xaa4-Xaa14;

Xaa5 is selected from: Asp, Gln, Glu, Arg, His and Lys;

Xaa6 is selected from: Pro and hydroxyproline;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Asn, Asp, Gln, Glu, Arg, His and Lys;

Xaa12 is selected from: Trp and Tyr;

Xaa13 is selected from: Cys, Met, Sec, Ser, Thr, Arg, His and Lys;

the N-terminus is optionally modified; and

the C-terminus is optionally modified.

2. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 1, wherein:

Xaa1 is absent;

Xaa2 is absent;

the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

Xaa5 is selected from: Asp, Gln and Glu;

Xaa6 is selected from: Pro and hydroxyproline;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Arg, His, and Lys;

Xaa12 is selected from: Trp and Tyr; and

Xaa13 is selected from: Cys, Met, Sec, Ser, and Thr.

3. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 1, wherein:

Xaa1 is absent;

Xaa2 is absent;

the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

Xaa5 is selected from: Arg, His and Lys;

Xaa6 is selected from: Pro and hydroxyproline;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Asn, Asp, Gln and Glu;

Xaa12 is selected from: Trp and Tyr; and

Xaa13 is selected from: Cys, Met, Sec, Ser and Thr.

4. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 1, wherein:

Xaa1 is absent;

Xaa2 is absent;

the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

Xaa5 is selected from: Arg, His and Lys;

Xaa6 is selected from: Pro;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Arg, His and Lys;

Xaa12 is selected from: Trp and Tyr; and

Xaa13 is selected from: Cys, Met, Sec, Ser, and Thr.

5. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 1, wherein:

Xaa1 is absent;

Xaa2 is absent;

the Xaa3-Xaa8 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

the Xaa4-Xaa14 linkage is selected from: a Cys-Cys linkage, a Sec-Sec linkage, a cystathionine linkage, a lactam bridge, a thioether linkage, and a dicarba linkage;

Xaa5 is selected from: Arg, His and Lys;

Xaa6 is selected from: Pro and hydroxyproline;

Xaa7 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa9 is selected from: Ala, Gly, Val, Leu and Ile;

Xaa10 is selected from: Arg and His;

Xaa11 is selected from: Arg, His, Lys, Asn, Asp, Gln and Glu;

Xaa12 is selected from: Trp and Tyr; and

Xaa13 is selected from: Cys, Met, Sec, Ser and Thr.

6. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 1, wherein the Xaa3-Xaa8 linkage and the Xaa4-Xaa14 linkage are independently selected from:

(i) a Cys-Cys linkage;

(ii) a Sec-Sec linkage;

(iii) a cystathionine linkage;

(iv) a lactam bridge,

(v) a thioether linkage, and

(vi) a dicarba linkage.

7. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 6, wherein the thioether linkage is a lanthionine linkage.

8. The muscle-type nicotinic acetylcholine receptor peptide antagonist of any one of claims 1-7, wherein the N-terminus is modified to comprise $C_1$-$C_6$ acyl, $C_1$-$C_8$ alkyl, $C_6$-$C_{12}$ aralkyl, $C_5$-$C_{10}$ aryl, $C_4$-$C_8$ heteroaryl, formyl, or a lipid.

9. The muscle-type nicotinic acetylcholine receptor peptide antagonist of any one of claims 1-8, wherein the C-terminus is modified to comprise $NH_2$, amino-acyl, amino-$C_1$-$C_8$ alkyl, amino-$C_6$-$C_{12}$-aralkyl, amino-$C_5$-$C_{10}$ aryl, amino-$C_4$-$C_8$ heteroaryl, or O-($C_1$-$C_8$ alkyl).

10. The muscle-type nicotinic acetylcholine receptor peptide antagonist of any one of claims 1-9, wherein a lipid is covalently attached to a cysteine, serine, lysine, threonine or tyrosine.

11. The muscle-type nicotinic acetylcholine receptor peptide antagonist of any one of claims 1-10, wherein the antagonist selectively inhibits a muscle-type nicotinic acetylcholine receptor.

12. The muscle-type nicotinic acetylcholine receptor peptide antagonist of claim 11, wherein the $IC_{50}$ is: about 1 millimolar to about 1 picomolar, less than about 200 nM, less than about 150 nM, or less than about 100 nM.

13. A cosmetic composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist of any of claims 1-12, for prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, in a subject.

14. The cosmetic composition of claim 13, formulated for topical use.

15. A pharmaceutical composition comprising the muscle-type nicotinic acetylcholine receptor peptide antagonist of any of claims 1-12, for: prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity; treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus, in a subject.

16. A method for preventing or temporarily improving the appearance in a subject for prevention or temporary improvement of the appearance of one or more of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity, comprising applying an effective amount of the cosmetic composition of claim 13 or 14 to the skin of the subject.

17. Use of the pharmaceutical composition of claim 15 in the manufacture of a medicament for preventing or temporarily improving one or more of the appearance of skin wrinkles, skin laxity, moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity, moderate to severe lateral canthal lines associated with orbicularis oculi activity, and moderate to severe forehead lines associated with frontalis muscle activity; treatment of overactive bladder (OAB); treatment of urinary incontinence; prophylaxis of headaches; treatment of spasticity; treatment of cervical dystonia; treatment of hypersalivation; treatment of blepharospasm associated with dystonia; or treatment of strabismus; in a subject, wherein the pharmaceutical composition of claim 15 is to be administered an effective amount to the subject.

18. The pharmaceutical composition of claim 15 for use as a therapy.